Technical Manual

Monoamine Oxidase (MAO) Activity Assay Kit

- Catalogue Code: MAES0021
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

16 – 641 U/L

Sensitivity:

16 U/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Monoamine oxidase (MAO, EC1.4.3.4) is a "flavin protein" containing the α-Mercaptopropionicacid in the outer membrane of mitochondria of cells. Its main function in vivo is to catalyze the metabolism of endogenous and exogenous monoamine substances. Under the action of MAO, monoamines are oxidized to produce deamination. MAO is ubiquitous in central nervous system and nerve endings, and it is mainly located on the outer membrane of mitochondria of tissues such as brain, liver and intestinal mucosa.

3. Intended Use

This kit can be used to measure monoamine oxidase (MAO) activity in serum, plasma and animal tissue samples.

4. Detection Principle

MAO can catalyze 4-dimethylambenzylamine to produce p-dimethylaminobenzaldehyde. p-Dimethylaminobenzaldehyde has a characteristic absorption peak at 355 nm. The activity of MAO can be calculated indirectly by analyzing the production of p-dimethylaminobenzaldehyde.

5. Kit components & storage

Item	Specification	Storage
Extraction Solution A	60 mL × 1 vial	2-8°C, 6 months
Extraction Solution B	60 mL × 2 vials	2-8°C, 6 months
Buffer Solution	60 mL × 2 vials	2-8°C, 6 months
Chromogenic Agent	5 mL × 1 vial	2-8°C, 6 months
UV Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (345-360 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

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6. Assay Notes:

1. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

2. UV microplate was used for detection.

3. During the tissue sample pre-treatment step, extraction working solution, extraction solution B and buffer working solution need to be pre-cooled for 30 minutes in advance.

4. During the operation steps, buffer working solution and chromogenic agent need to be pre-heated at 37°C for 30 min in advance.

5. For tissue sample, the protein concentration in sample should be determine separately.

7. Reagent preparation:

1. The preparation of **extraction working solution:** Mix extraction solution A with double distilled water fully at a ratio of 1:1. The prepared solution can be stored at 2-8°C for 1 month.

2. The preparation of **buffer working solution:** Mix buffer solution with double distilled water fully at a ratio of 1:1. The prepared solution can be stored at 2-8°C for 1 month.

8. Sample Preparation

1. Serum (plasma): detect directly.

2. 10% tissue homogenate: Accurately weigh the tissue sample, add 9 times the volume of pre-cooled extraction working solution according to the ratio of weight (g): volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 1000 g for 10 min (Note: determine the protein concentration of supernatant (E-BC-K318-M) before centrifugation), then take the supernatant and centrifuge at 10000 g at 4°C for 30 min, discard the supernatant and keep the sediment. Add 1 mL of pre-cooled extraction solution B and mix fully, centrifuge at 16,000 g at 4°C for 40 min, discard the supernatant and keep the sediment. Finally, add 1 mL of pre-cooled buffer working solution, mix fully, and preserve it on ice for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (16 – 641 U/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
10% Mouse liver tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
Human serum	1

Note: The diluent is buffer working solution;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 355 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

Note: S1-S96, sample wells.

10. Operation Steps

1. **Sample tube:** Add 100 μL of sample to 1.5 mL tube.
   Blank tube: Add nothing.

2. **Sample tube:** Add 800 μL of buffer working solution to the tube.
   Blank tube: Add 1000 μL of buffer working solution to the tube.

3. **Sample tube:** Add 100 of chromogenic agent to sample tube.
   Blank tube: Add nothing.

4. Set the spectrophotometer to zero with blank tube and measure the OD value of sample tube with 1 cm optical path cuvette at 355 nm, recorded as A_1, and then incubate accurately at 37°C for 30 min, measure the OD values of each tube again, recorded as A_2.

Operation Table

	Blank tube	Sample tube
Sample (μL)	100	
Buffer working solution (μL)	800	1000
Chromogenic agent (μL)	100	
Set the spectrophotometer to zero with blank tube and measure the OD value of sample tube with 1 cm optical path cuvette at 355 nm, recorded as A_1, and then incubate accurately at 37°C for 30 min, measure the OD values of each tube again, recorded as A_2.		

11. Calculations

1. Serum (plasma) and other liquid sample:

Definition: the amount of enzyme in 1 L of sample that catalyze the substrate to produce 1 nmol p-dimethylaminobenzaldehyde at 37 ℃ for 1 min is defined as 1 unit.

$$\text{MAO activity (U/L)} = \frac{(A_2 - A_1)}{\varepsilon \times d} \times V_1 \div V_2 \div T$$

2. Tissue sample:

Definition: the amount of enzyme in 1 g of tissue protein that catalyze the substrate to produce 1 nmol p-dimethylaminobenzaldehyde at 37 °C for 1 min is defined as 1 unit.

$$\text{MAO activity (U/gprot)} = \frac{(A_2 - A_1)}{\varepsilon \times d} \times V_1 \div (V_2 \times C_{pr}) \div T$$

T: the time of incubation in the reaction, 30 min
ε: the molar extinction coefficient of p-dimethylaminobenzaldehyde, 2.77×10^{-4} L/(nmol·cm)
d: the optical path of cuvette, 0.6 cm
V_1: the total volume of reaction, 200 μL
V_2: the volume of sample added to the reaction, 25 μL
C_{pr}: Concentration of protein in sample, gprot/L.

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12. Performance Characteristics

Detection Range	16 – 641 U/L
Sensitivity	16 U/L
Average recovery rate (%)	105
Average inter-assay CV (%)	6.0
Average intra-assay CV (%)	3.3

Analysis

For rat liver tissue, take 25 μL of 10% rat liver tissue homogenate, carry the assay according to the operation table.

The results are as follows:

the initial OD value of the sample (A_1) is 0.684, the OD value of the sample after 30 min (A_2) is 1.016, the concentration of protein in sample is 11.27 gprot/L, and the calculation result is:

MAO activity (U/gprot)

$$= (1.016-0.684) \div (2.77\times10^{-4}) \div 0.6 \times 200 \div (11.27\times25) \div 30$$

$$= 47.26 \text{ U/gprot}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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